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Detection of carbon disulfide in breath and air: a possible new risk factor for coronary artery disease

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Summary. Carbon disulfide (CS_2) is toxic to the heart and arteries; chronic exposure can result in accelerated atherosclerosis and coronary artery disease in humans and animals. Exposure to CS_2 was investigated in normal volunteers working in New York City, using a new and highly sensitive assay. Volatile organic compounds in breath and air were captured in adsorbent traps containing graphitized carbon and molecular sieve, then thermally desorbed, concentrated by two-stage cryofocusing, and assayed for CS_2 by gas chromatography/mass spectroscopy. Breath CS_2 assays were performed in 42 normal volunteers, as well as in room air and in outdoor air collected at sites in and around New York City. The assay was linear, reproducible, and sensitive to picomolar (10^{-12} mol/l) quantities. CS_2 was detected in all samples of breath and indoor and outdoor air (mean concentrations were 5.25 pmol/l, SD = 3.89 in breath, 8.26 pmol/l in indoor air, and 3.92 pmol/l in outdoor air) (NS). The alveolar CS_2 gradient (alveolar − inspired CS_2) ranged from −20.0 to 8.0 nmol/l, separating subjects into either "excreters" or "retainers" of CS_2. In view of the known toxicity of CS_2, atmospheric pollution with CS_2 merits attention as a possible new risk factor for the development of accelerated atherosclerosis and coronary artery disease.

Key words: Air assays – Atherosclerosis – Breath assays – Carbon disulfide – Coronary artery disease

Introduction

Carbon disulfide (CS_2) is a toxic chemical which can cause accelerated atherosclerosis and coronary artery disease [1–3]. The toxicity of CS_2 was first recognized during the nineteenth century, when it was widely employed in industry as a solvent to soften rubber. Exposed workers in poorly ventilated factories frequently developed neurotoxicity, florid psychoses, and lesions in the heart and arteries known then as "vasculopathia sulfocarbonica" [1, 3]. The cardiovascular toxicity of CS_2 was confirmed in 1968, when Tiller et al. reported that the death rate from coronary heart disease was more than doubled in workers who had been chronically exposed to high levels of CS_2 in viscose rayon factories [4]. Similar findings have subsequently been reported in epidemiologic studies of industrial workers exposed to CS_2 in Finland [5, 6], Britain [7], the Soviet Union [8] and the United States [9]. CS_2 toxitcity has also been implicated as a cause of hypertension, retinal angiopathy, hyperlipidemia, and altered carbohydrate metabolism [3]. The epidemiologic link between exposure to CS_2 and coronary artery disease in humans has been supported by the results of toxicity studies in animals: CS_2 causes arterial lesions in rats and rabbits and enhances the formation of atheroma in animals fed a diet high in lipids [3, 10–12]. In addition, CS_2 is toxic to the myocardium and may precipitate lethal arrhythmias [13].

Factory workers exposed to CS_2 account for only a small fraction of all sufferers from coronary artery disease. However, these findings have provoked speculation that a much larger population may be at risk. Schilling [2] observed that the air of industrialized countries is frequently polluted with sulfur compounds and suggested that air pollution should be investigated as a possible cause of coronary artery disease. However, no previously published studies have supported this hypothesis, possibly because it is technically difficult to detect low levels of CS_2 exposure in humans.

Most published methods for the detection of CS_2 exposure in humans have utilized breath tests, which are noninvasive and are based on well-understood physiologic mechanisms. CS_2 is a highly volatile compound which is rapidly absorbed or excreted through the lungs depending upon the concentration gradient across the pulmonary alveolar membrane [14, 15]. CS_2 in the blood is mainly confined to the erythrocytes [16] and equilibrates with CS_2 in the alveolar air by passive diffusion across the alveolar membrane [17, 18]. The concentration of CS_2 in the alveolar breath varies with the vapor pressure of CS_2 in the blood of subjects exposed to atmospheric CS_2 [14, 19], and in patients treated with disulfiram, a precursor of CS_2 [20–22]. However, no previous studies

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have reported the presence of CS_2 in human breath in the absence of a history of disulfiram ingestion or of known prior exposure to CS_2, even when an assay sensitive to a concentration of 10^{-7} mol/l was used [22].

Recently, a more sensitive assay using gas chromatography (GC) for micronanalysis of volatile compounds in the breath has been reported, in which one compound observed in normal human breath eluted with a retention time similar to CS_2 [23]. The identity of the compound was not confirmed by mass spectroscopy (MS). This report describes an improvement in the assay using combined GC/MS, capable of detecting CS_2 in the breath in picomolar concentrations (10^{-12} mol/l). This highly sensitive and specific assay was employed to investigate the presence of CS_2 in the breath of normal human volunteers as well as in the air surrounding New York City.

Materials and methods

Collection of volatile compounds in the breath. The method has been described [23]. A pump-assisted device was used to collect 20-l samples of breath. Water was extracted by passing the breath sample through anhydrous calcium sulfate, and the volatile compounds were then captured by adsorption to graphitized carbon and molecular sieve in a stainless steel trap.

Desorption of volatile compounds. The method has been described [23]. The stainless steel trap containing the sample was transferred to a microprocessor-controlled automatic desorber and concentrator. The volatile compounds were desorbed at 300°C and flushed in a stream of helium to two successive cryofocusing traps cooled to −150°C. The enriched sample, now concentrated nearly 300 000 times, was then injected into a gas chromatograph.

GS/MS assay of volatile compounds. The organic compounds in the concentrated breath sample were separated in a gas chromatograph with subambient temperature programming capability (Model HP5890A, Hewlett Packard, Avondale, PA). Following the splitless injection of the sample from the cryofocusing trap, the oven temperature was held at 0°C for 10 min, then increased by 7°/min for 40 min, and finally held at 280°C for 10 min. A 50-m "007" series fused silica capillary column was used, with 0.32 mm ID and 6.0-μm methyl silicone bonded phase (# 81123C, Quadrex Corp., New Haven, CT). The carrier gas was ultrapure helium flowing at 4.5 ml/min. The column effluent was transferred at 280°C to a mass spectrometer (Model 700 ion trap detector, Finnigan MAT, San Jose, CA) with the following acquisition parameters: full scan acquisition mode, scan range 10–650 amu, scan time 1.000 s. The detector signal was fed to a microcomputer (IBM PS2 Model 30286) for analysis and storage.

Calibration of CS_2 assay. A CS_2 vapor standard (195.2 pmol/ml) was prepared by the method of Morris et al. [24] using pure CS_2 (Fisher Scientific, Springfield, NJ) diluted 1:200 in methanol and volatilized at 65°C in ultrapure helium. A standard curve was obtained by loading duplicate samples of the vapor standard (0, 0.05, 0.12, 0.25, 0.5, 1.0, and 2.0 ml) onto adsorbent traps similar to those used in the breath-collecting apparatus. In order to load a sample, the trap was sealed in an airtight metal container (air sampling adapter 16-1686-000, Tekmar, Cincinnati, OH) and the standard was injected through a silicone-rubber septum. Each trap was then assayed by GS/MS as described above; the CS_2 peak was identified by its elution time and mass spectrum, and quantified by the area under the curve (AUC). The correlation between the quantity of CS_2 in the sample and the resulting AUC was determined by linear regression. The precision and accuracy of the assay were estimated by intraassay and interassay batch variation.

Intraassay batch variation was determined by loading 2.0-ml samples or vapor standard ($n = 8$) onto adsorptive traps which were then assayed as described above during a single working day. Since only one assay could be performed at a time, and each assay required more than 1 h of instrument time, eight samples represented a maximal daily batch load. The amount of CS_2 detected in each sample was determined from the standard curve, and the coefficient of variation (CV) was calculated. Interassay batch variation was determined in the same fashion, except that eight samples were assayed two at time over four sequential working days.

Human studies. Forty-two normal volunteers in good general health were recruited from the staff and students of St. Vincent's Medical Center of Richmond. All volunteers were asked about their tobacco smoking history, but were not requested to fast or to modify their dietary or smoking behavior. All breath samples were collected between 8.00 and 11.00 a.m. in a hospital office, and assayed as described above. All volunteers gave their signed informed consent to participate in this research, which was approved by the Institutional Review Board of St. Vincent's Medical Center of Richmond.

Air assays. Background samples of the room air (20 l) were obtained at the time of all breath collections. Air samples were also obtained at six randomly selected sites in and around New York City in order to exclude the possibility that the CS_2 observed in the breath and air samples might have originated from pollution confined to Staten Island. Duplicate field collections of 20 l outdoor air were performed in a similar fashion to the indoor air collections, using a portable microprocessor-controlled air sampling pump (model 224-PCXR3, SKC Inc, Eighty Four, PA).

Analysis of data. The concentrations of CS_2 in the breath of male and female smokers and non-smokers, room air, and outdoor air, were compared by one-way analysis of variance and the Student-Newman-Keuls multiple-range test. In addition, the alveolar CS_2 gradient (alveolar CS_2 minus inspired CS_2) was determined for each subject.

Results

Calibration of CS_2 assay

The assay was sufficiently sensitive to detect 9.76 pmol CS_2 in a standard, a quantity equivalent to the amount of

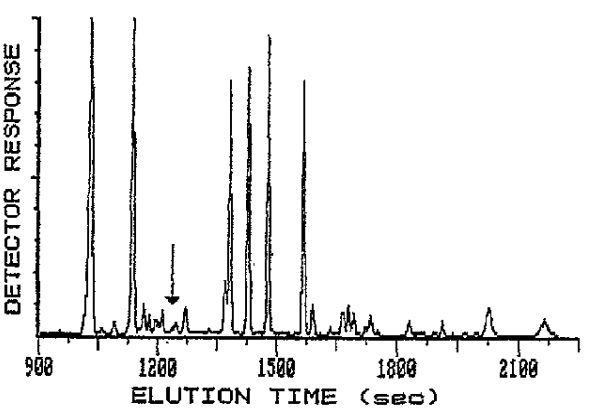

Fig. 1. Chromatogram of human breath. The assay of a typical breath sample is shown, using GC combined with ion trap detection. The *arrow* indicates the peak which eluted at the same time as pure CS_2

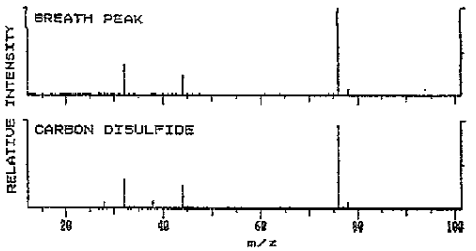

Fig. 2. Mass spectra of CS_2 and the breath peak with the same elution time as CS_2. The ion trap detector generated a mass spectrum for every compound in the breath, which yielded a peak in the chromatogram shown in Fig. 1. The *upper panel* demonstrates the mass spectrum of the breath peak with the same elution time as CS_2. The *lower panel* demonstrates the mass spectrum obtained from a pure CS_2 standard. *m/z* is the ratio of mass to charge of the molecular fragments detected in the ion trap

Table 1. CS_2 levels in breath and air

	No. of observations	Mean age (years)	SD	Mean CS_2 concentration (pmol/l)[a]	SD[a]
Male smokers	8	35.7	4.82	4.80	3.19
Male nonsmokers	12	32.9	6.44	6.32	5.24
Female smokers	12	36.8	9.34	5.79	3.49
Female nonsmokers	10	34.5	9.20	3.67	2.79
All volunteers	42	34.9	7.68	5.25	3.89
Room air	9			8.26	5.58
Outdoor air	6			3.92	0.63

[a] No significant differences observed between any of the groups

CS_2 contained in a 20-l sample of breath or air with a concentration of approximately 0.5 pmol/l. The response was linear ($y = 57.69\ x - 208.94$, $r^2 = 0.99$, where y = AUC and x = pmol CS_2 in the sample) (intraassay CV = 14.14%, interassay CV = 11.60%).

Assay of breath and air

Several peaks were observed in the chromatograms of the breath samples obtained from the normal volunteers, and all samples demonstrated a peak with the same elution time as pure CS_2 (Fig. 1). In every case, the mass spectrum of this breath peak was similar to the mass spectrum of pure CS_2 (Fig. 2), confirming that CS_2 was present in all of the samples of human breath. Similarly, CS_2 was detected in all samples of indoor air as well as in all samples of outdoor air collected in and around New York City (Tables 1, 2). No significant differences in the breath concentrations of CS_2 were observed between male and female smokers and nonsmokers, or between the concentrations of CS_2 in room air, outdoor air, and breath (Table 1). Alveolar CS_2 gradients ranged from −20.0 to 8.0 pmol/l in an approximately bell-shaped curve of frequency of distribution (Fig. 3).

Table 2. CS_2 in outdoor air at sites in and around New York City

Collection site	CS_2 (pmol/l)
Empire State Building (observation deck)	4.01
Times Square	3.98
Bayley Seton Hospital, Staten Island (roof)	4.61
New Jersey Turnpike (Newark Airport toll plaza)	4.13
Central Park (West 85th Street entrance)	4.08
Brighton Beach boardwalk, Brooklyn	2.72

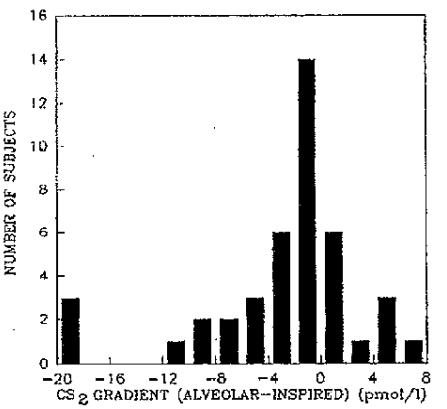

Fig. 3. Frequency distribution of the alveolar CS_2 gradient. The alveolar CS_2 gradient was the difference between the concentration of CS_2 observed in the breath and in the inspired air, and reflects the vapor pressure gradient across the pulmonary alveolar membrane. Subjects were termed "excreters" or "retainers," according to whether their alveolar CS_2 gradient was negative or positive

Discussion

All of the normal volunteers in this study had been exposed to CS_2, as shown by the presence of CS_2 in their breath and in the air they were breathing. Assays of the outdoor air at six randomly selected sites demonstrated that air pollution with CS_2 was not confined to Staten Island, where the breath samples were collected. No statistically significant differences were observed between the concentrations of CS_2 in room air, outdoor air, and the breath of male and female smokers and nonsmokers.

Carbon disulfide in blood and air equilibrates rapidly across the pulmonary alveolar membrane, so that the vapor pressure exerted by CS_2 in the alveolar breath and that in the pulmonary capillary blood are approximately equal [14–18]. Consequently, the alveolar CS_2 gradient (alveolar CS_2 minus inspired air CS_2) appears to reflect the gradient between the vapor pressure of CS_2 in the inspired air and in the pulmonary arterial blood. The frequency distribution curve of the alveolar CS_2 gradient (Fig. 3) was of particular interest, since it demonstrated that normal subjects could be divided into two groups according to whether the vapor pressure of CS_2 in the blood was higher or lower than the vapor pressure of CS_2 in the inspired air. The majority of subjects exhibited a nega-

tive alveolar CS_2 gradient, i.e., the vapor pressure of CS_2 was lower in the blood than in the inspired air. This finding is consistent with what is known of CS_2 metabolism in humans: CS_2 is excreted through the kidneys as well as the lungs, either unchanged or in the form of urinary thio compounds, including glutathione conjugates, mercapturic acids, and other sulfur-containing metabolites [3]. Those subjects with a negative alveolar CS_2 gradient appeared to be "excreters" – i.e., they apparently excreted part of the inhaled CS_2 load through extrapulmonary pathways as well as through the lungs. On the other hand, subjects with a positive alveolar CS_2 gradient appeared to be "retainers" who were unable to dispose of the environmental CS_2 load with the same efficiency as the "excreters." It is not yet clear how CS_2 metabolism differs between the "excreters" and the "retainers"; however, the existence of these differences suggests that there would be wide variations in the blood levels of CS_2 within a population breathing the same polluted air, with a consequent wide spectrum of any toxic effects that might result from this exposure. Further studies will be needed to determine wheter these findings can be replicated beyond the confines of the boroughs of New York City.

The atherogenic effects of CS_2 were originally described in humans and animals exposed to higher ambient concentrations than those observed in this study. Most industrialized countries now have regulated standards for human exposure to CS_2; in the United States, the NIOSH recommendation is that exposure should not exceed 1 part per million (ppm) as a 10-h total weighted average in a 40-h weeks, or 10 ppm for any 15-min period (1 ppm of CS_2 is approximately equivalent to a concentration of 1.66×10^{-5} mol/l). In other industrialized countries, the theshold limit value ranges from 4 to 20 ppm [3]. However, Ruijten et al. [25] found progressive peripheral neuropathic changes in subjects who had been exposed to CS_2 over a period of 40 years at levels that had previously been considered safe for industrial workers [25]. Their findings, and the results of other epidemiologic studies of industrial workers, have generally supported the conclusion that CS_2 acts as a cumulative slow poison. It is not known whether there is any threshold level of exposure to CS_2 which is safe for humans.

Inhaled CS_2 may interact with serum lipids to promote the formation of atheromatous plaque. An elevated serum level of low density lipoprotein (LDL) is a risk factor for the development of coronary artery disease [26], and Laurman et al. have shown that CS_2 interacts with LDL in vitro, decreasing the number of free amino groups in apolipoprotein B100 [27]. The CS_2-modified LDL is catabolized by the "scavenger" receptor of monocyte-derived macrophages, resulting in the formation of foam cells laden with cholesteryl esters, a process believed to be involved in the development of atherosclerotic plaques. It appears likely that plaque formation would be accelerated by the interaction between inhaled CS_2 and circulating LDL.

The prevalence of coronary artery disease has risen and fallen in parallel with the quantity of volatile organic sulfur compounds discharged into the air by industrial pollution. Acute myocardial infarction had been recognized only rarely before Herrick's classic description in 1912 [28], and Harrison and Reeves observed: "Most medical students and house officers of the 1920-to-1925 era never heard of the disease during their period of clinical training" [29]. Over the next four decades, coronary heart disease emerged from clinical obscurity to become the leading cause of death in the United States [30] as well as in many other Western industrialized countries. This surge in prevalence coincided with the aging of the first generation that had been exposed to air which was heavily polluted by smoke derived from sulfur-containing fuels. Between 1850 and 1910, energy consumption in the United States increased more than eightfold, from less than 0.5×10^{15} kcal/year to more than 4.0×10^{15} kcal/year. Most of this energy was derived from burning coal and petroleum, yielding smoke rich in volatile organic sulfur compounds. Over the next 50 years, energy consumption tripled again, to nearly 12.0×10^{15} kcal/year [31]. This period, from 1910 to 1960, was accompanied by a dramatic increase in both air pollution and death from coronary artery disease. Many governmental responses to the problem of worsening air pollution (particularly the Clean Air Act of 1963 and state and local ordinances to restrict automotive emissions) data from the early 1960s; subsequent improvements in the quality of the air have coincided with a major reduction in mortality from coronary heart disease [32].

In a 1970 editorial on "Coronary heart disease in viscose rayon workers," Schilling concluded: "Since there are sulfides in cigarette smoke, and sulfur compounds pollute our atmosphere, it is worthwhile investigating the possibility that these may be significant causal factors in coronary heart disease" [2]. This study has shown that CS_2 can be detected in the air of New York City, as well as in the breath of normal inhabitants. Environmental exposure to CS_2 in polluted air merits further investigation as a possible new risk factor for the development of accelerated atherosclerosis and coronary artery disease.

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